FEBS Letters 589 (2015) 897–903

FEBS Letters

journal homepage: www.FEBSLetters.org

Msi1 confers resistance to TRAIL by activating ERK in liver cancer cells

Nianli Liu [a,b], Tianran Chen [a], Xiaohong Wang [a], Darong Yang [a], Binbin Xue [a], Haizhen Zhu [a,b,*]

[a] Department of Molecular Medicine, College of Biology, State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, China
[b] Research Center of Cancer Prevention & Treatment, Translational Medicine Research Center of Liver Cancer, Hunan Provincial Tumor Hospital (Affiliated Tumor Hospital of Xiangya Medical School of Central South University), Changsha 410013, China

ARTICLE INFO

Article history:
Received 20 December 2014
Revised 3 February 2015
Accepted 23 February 2015
Available online 3 March 2015

Edited by Quan Chen

Keywords:
Msi1
Tumor necrosis factor-related apoptosis-inducing ligand
Drug resistance
Hepatocellular carcinoma
ERK

ABSTRACT

To investigate TRAIL resistance mechanisms in hepatocellular carcinoma (HCC), we isolated a stable TRAIL-resistant sub-population of the HCC cell line LH86, designated LH86-TR. Differential activation of AKT was not responsible for acquisition of TRAIL resistance. Cells with both congenital and acquired resistance to TRAIL exhibited increased Msi1 expression, which conferred TRAIL resistance by activating ERK. Forced expression of Msi1 decreased the sensitivity of HCC cells to TRAIL both in vitro and in vivo. Conversely, shRNA-mediated depletion of Msi1 enhanced TRAIL efficacy. SiRNA-mediated depletion of ERK overcame TRAIL resistance. Hence, we conclude that Msi1 is a mediator of TRAIL resistance in HCC cells.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and one of the leading causes of cancer-related death worldwide [1]. Existing treatment modalities such as surgery, radiofrequency ablation and chemotherapy have demonstrated limited therapeutic efficacy. Developing new cancer therapeutic strategies is particularly important in human HCC. TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) displays potent anticancer effects in a wide range of cancers resistant to conventional therapy without apparent toxic side effects to normal cells [2]. Binding of homotrimeric TRAIL to DR4 and DR5 induces oligomerization of the receptors and promotes formation of the death inducing signaling complex (DISC), resulting in activation of the initiator caspase-8. In type I cells, death receptor-initiated caspase-8 activation generates a signal strong enough to activate downstream effector caspase-3 that triggers execution of apoptosis. In contrast, in type II cells, the magnitude of caspase-8 activation is not sufficient to directly activate caspase-3. Active caspase-8 cleaves Bid, which induces activation of Bax, followed by permeabilization of mitochondria. Pro-apoptotic proteins such as cytochrome *c*, Smac and DIABLO are released from mitochondria, activating caspase-9 and caspase-3 [3]. Several TRAIL-based novel drugs targeting its receptors are in the preclinical or clinical development [4]. Nevertheless, resistance to TRAIL-induced apoptosis in cancer cells is one impediment to the use of TRAIL-based agents as antitumor drugs. Though many cancer cells are sensitive to TRAIL-induced apoptosis congenitally, they can acquire TRAIL resistance after long-time treatment by TRAIL.

Msi1 is a highly conserved RNA-binding protein that serves as a stem cell marker in central nervous system. In neural stem cells, Msi1 activates Notch signal and regulates neural stem cell self-renew [5]. In a medulloblastoma cell line Daoy, a subset of cell proliferation, differentiation and survival genes belong to Notch, Hedgehog and Wnt pathway are differently expressed after Msi1 knockdown [6]. Msi1 increases the growth and survival of many glioma cell lines by promoting the activations of both Notch and PI3K/AKT signaling [7]. ERK phosphorylation is increased in Msi1 overexpressing COMMA cells, associated with increasing PLF1 secretion and reduction of DKK3, promoting proliferation of COMMA cells [8]. These findings suggest that Msi1 plays a pivotal role in stem cell maintenance, nervous system development, and tumorigenesis. The relationship between Msi1 functions and HCC progresses is also studied recently. Msi1 is elevated in the selected

☆ Author contributions: H.Z. conceived the experiments. H.Z. and N.L. designed the experiments. N.L. carried out the main experiments and analyzed the data. T.C., X.W., B.X. and D.Y. helped the experiments. N.L. and H.Z. wrote the paper. All authors read and approved the final manuscript.

* Corresponding author at: Department of Molecular Medicine, College of Biology, State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, China. Fax: +86 731 88821385.
E-mail address: zhuhaizhen69@yahoo.com (H. Zhu).

http://dx.doi.org/10.1016/j.febslet.2015.02.026
0014-5793/

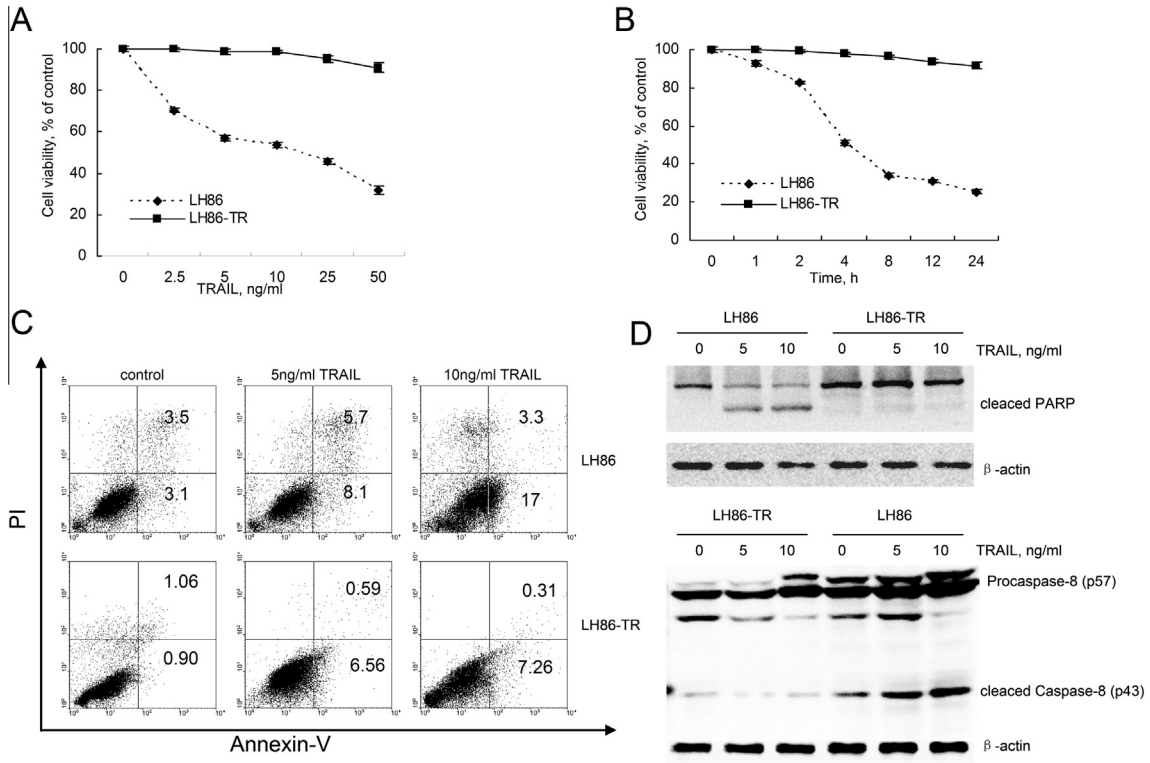

Fig. 1. Generation of TRAIL-resistant LH86-TR liver cancer cells. (A and B) TRAIL-dependent loss of viable cells, measured by the MTT assay. The data represent the mean and standard deviation of experiments performed in triplicate. (C) Apoptosis was confirmed by Annexin-V/PI staining following exposure to TRAIL for 3 h. (D) Immunoblots of PARP showed the full length 116-and 89-kDa apoptosis-related cleaved fragments in the parental LH86 exposed to TRAIL, confirming apoptotic cell death induced by TRAIL.

sorafenib-resistant HCC cells and enhances cellular migration and invasion with activated EMT process [9]. Msi1 modulates proliferation of HCC cells by inhibiting APC and DKK1 mRNA translation [10]. Whether Msi1 regulates TRAIL sensitivity of HCC cells is still unexplored.

Here we selected a HCC cell line LH86-TR, acquired resistance to TRAIL, from a TRAIL-sensitive cell line LH86. Various oncogenes and tumor suppressor genes tested in these two cell lines did not express differently. Interestingly, p-AKT was decreased in LH86-TR cells compared to LH86. Inhibition of AKT only marginally improved TRAIL-induced apoptosis in acquired resistant HCC cells. By comparing the expression of the stem cell markers between LH86 and LH86-TR cells, we found that Msi1 expression was increased in TRAIL-resistant cells. We also found that Msi1 conferred TRAIL resistance by activating ERK. Inhibition of ERK could sensitize HCC cells to TRAIL.

2. Materials and methods

2.1. Cell lines

Human HCC cell lines Huh7, LH86, HLCZ01 were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum at 37 °C in 5% CO_2 [11].

2.2. Generation of TRAIL-resistant cells

We established a TRAIL-resistant cell line by exposing LH86 cells to increasing concentrations of TRAIL (from 5 ng/ml to 50 ng/ml). After 3 months selection, a TRAIL-resistant LH86-TR cell line, able to grow in the presence of 50 ng/ml TRAIL, was isolated. LH86-TR cells were maintained in DMEM in 10% fetal bovine serum with continuous exposure to 10 ng/ml TRAIL.

2.3. Reagents and antibodies

Mouse anti-caspase-9, anti-caspase-3, anti-Bax, anti-AKT, anti-pAKT(473) and anti-PARP antibodies were purchased from Cell Signaling Technology. Mouse anti-β-actin, anti-Bcl-xL and anti-FLAG monoclonal antibodies were from Sigma. Anti-Msi1 antibody was from Abcam. The antibodies anti-Bid, anti-PTEN, anti-K-RAS, anti-p38, anti-ERK1 and p-ERK1/2 were obtained from Sangon. Anti-pSTAT3(705) and anti-NOXA antibodies were from SANTA CRUZ. Anti-caspase-8 and anti MCL-1 antibodies were from Boster. The Annexin V-FITC apoptosis detection kit and TRAIL were purchased from Merck Millipore and R&D Systems respectively.

2.4. Overexpression and knock down experiments

pENTER-Msi1 with C terminal Flag and His tag plasmid was purchased from Vigene. The oligonucleotides encode 19-mer sequence specific to the Msi1 mRNA were incorporated into the pSilencer-neo plasmid (Ambion). The sequences of Msi1 shRNAs targeting two regions of Msi1 were 5′-AATCGTTCGAGTCACCATCTT-3′ and 5′-AATAACTCCGGCTGGCGTA GG-3′. The siRNA sequence for ERK1 was 5′-TTAGGTAGGTCATCCAGCT-3′. All constructs were confirmed, and transfected into cells using Lipofectamine 2000 reagents (Invitrogen).

2.5. Western blot analysis

Cell lysates were prepared on ice, and protein concentrations were determined using the BCA assay (Dingguo). Cell lysates were separated on SDS–page gel and transfected to PVDF membrane (Bio-Rad). Proteins were detected with the appropriate primary antibody, horseradish peroxidase-linked secondary antibodies, and visualized by chemiluminescence with the West Pico system (Thermo).

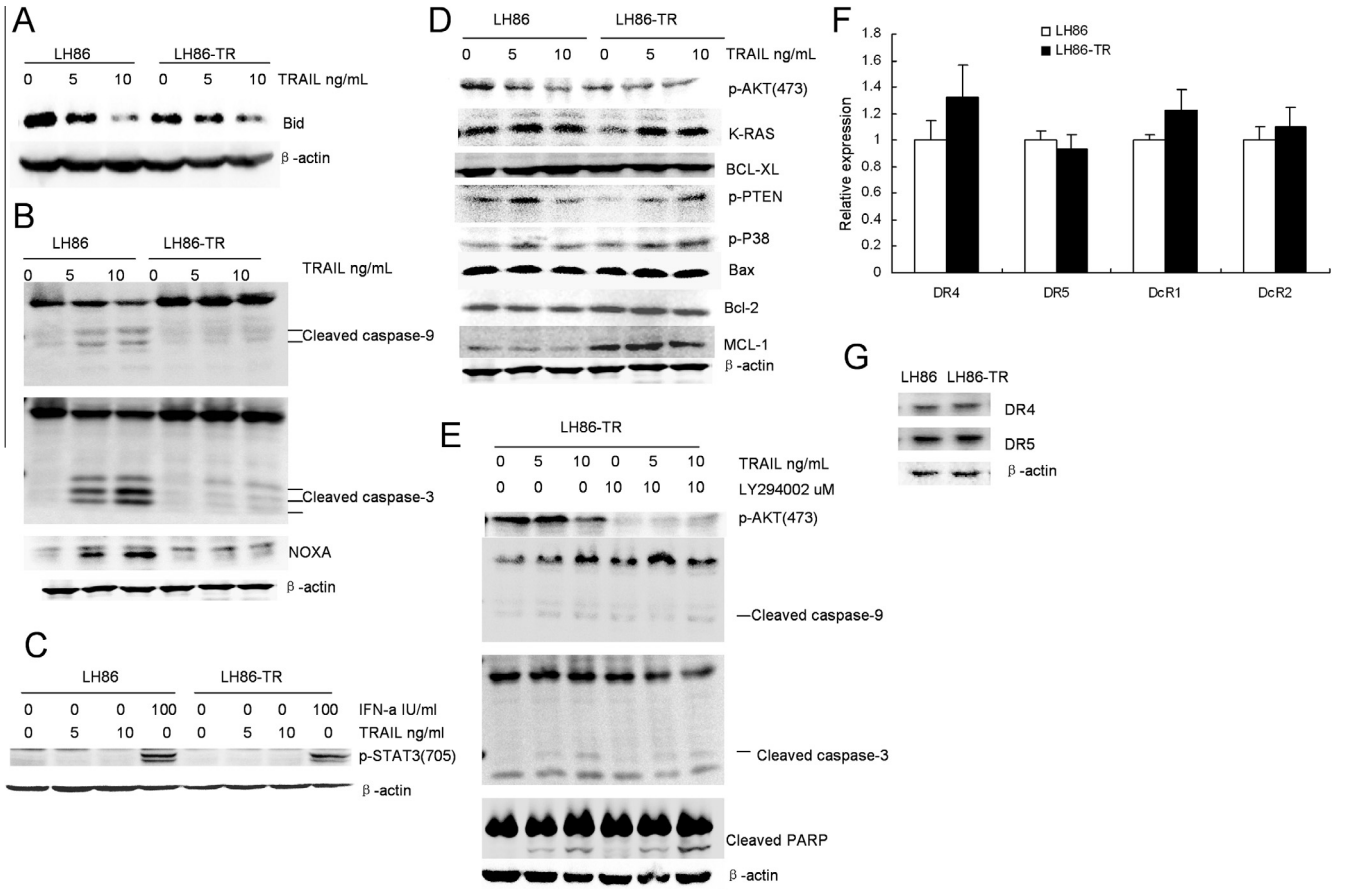

Fig. 2. Differential regulation of pro-survival signals is not responsible for the acquisition of TRAIL resistance in LH86-TR. (A and B) LH86-TR and parental LH86 cells were treated by TRAIL. Bid, cleavage form of caspase-9, cleavage form of caspase-3 and Noxa were determined by Western blot assay. (C and D) Parental LH86 and TRAIL-resistant LH86-TR was treated with TRAIL and modulation of pro-survival and apoptotic signaling was compared by Western blot analysis. (E) AKT inhibitor LY294002 (10 μM) failed to overcome resistance to TRAIL as determined by Western blot of PARP, caspase-9 and caspase-3 cleavage. (F) TRAIL receptors DR4, DR5, DcR1, DcR2 mRNA levels in LH86 and LH86-TR cells were determined by real-time PCR. Error bars represent S.D. from triplicate experiments. (G) DR4 and DR5 proteins were analyzed by Western blot.

2.6. Quantitative real-time PCR

Total cellular RNA was extracted using TRZIOL reagent according to the manufacturer's protocol. One microgram of cellular RNA was reverse-transcribed in a total volume of 20 μl using the one-step script RT kit (Invitrogen). The primers targeted DR4, DR5, DcR1, DcR2 have been reported previously [11]. The primers targeted Msi1, CD133, SOX2, Oct4 have been described [9]. The internal control was GAPDH. Fold variations were calculated after normalization to GAPDH.

2.7. Flow cytometry analysis

LH86 and LH86-TR cells were treated with TRAIL for the indicated hours. Apoptosis was determined by staining cells with annexin V-FITC and propidium iodide according to the manufacturer's protocol (Merck). Samples were analyzed by using the FACScan flow cytometer (Beckman).

2.8. In vivo assay

Animal studies were performed in accordance with protocols approved by the Institutional Laboratory Animal Care and Use Committee of Hunan University. Stable-Msi1 expressing LH86 cells and stable-Msi1 knockdown LH86-TR cells were selected with antibiotics for 2 weeks. Nude mice were inoculated subcutaneously with 5×10^6 tumor cells/mouse and the tumors were allowed to grow to about 100 mm^3. TRAIL (10 mg/kg/day) or vehicle (PBS) was administered by tail vein injection every 3 days. Tumors were measured using calipers and their volumes calculated using the following standard formula: $width^2 \times length \times 0.52$.

2.9. Statistical analysis

Statistical analyses were performed with the two-tailed Student's *t*-test, and error bars represent S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Generation of stable TRAIL-resistant human liver cancer cells

The human HCC cell line LH86 was used as a model to investigate the mechanism of acquired TRAIL resistance because these cells are relatively susceptible to TRAIL-induced cytotoxicity [11]. We obtained TRAIL-resistant stable cells by subjecting LH86 cells to repeated exposure to TRAIL. Exposure of LH86 cells to TRAIL resulted in a dose- (Fig. 1A) and time-dependent (Fig. 1B) reduction in cell viability. Residual surviving cells were propagated with periodic exposure to TRAIL to yield a TRAIL-resistant stable cell line, LH86-TR. As shown in Fig. 1A and B, less than 10% loss of viable cells could be detected in LH86-TR cells after exposure to 50 ng/ml TRAIL for 24 h, in contrast to 75% loss of viable cells observed in LH86 cells. To determine the nature of cell death induced by

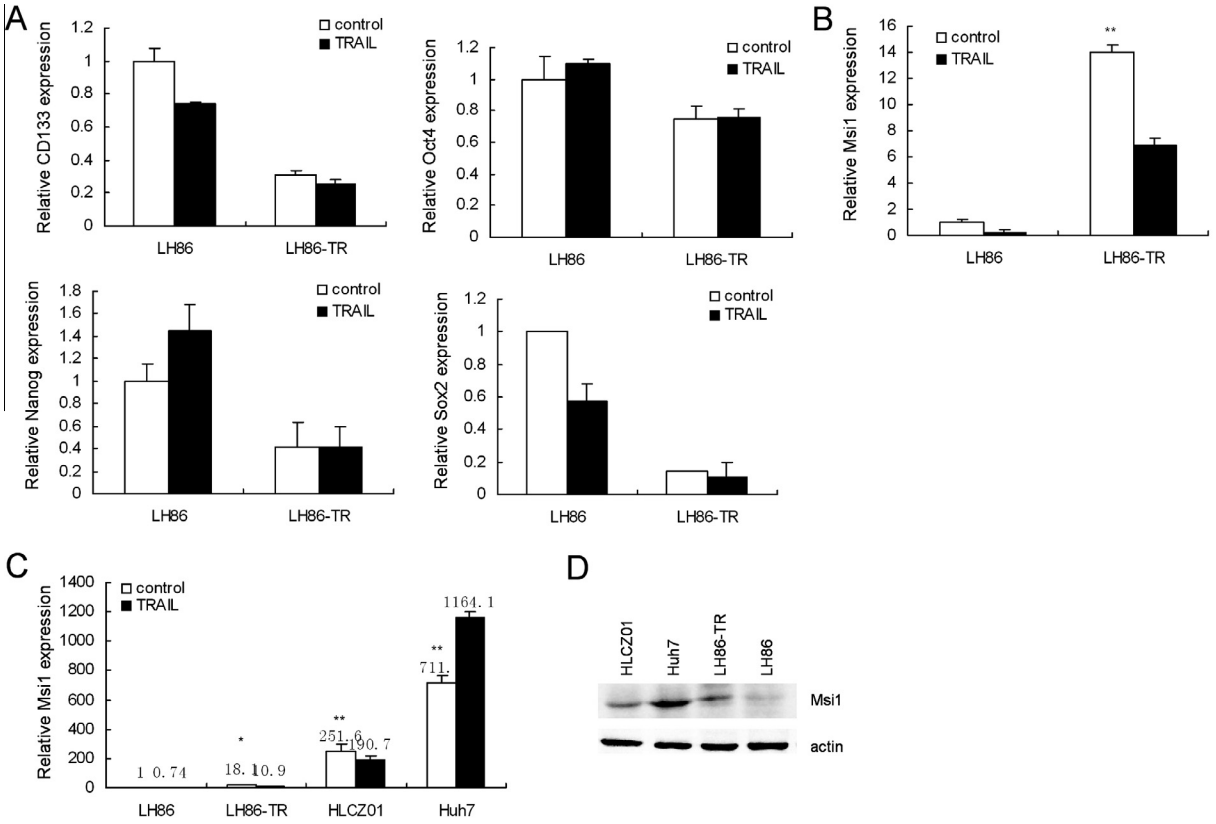

Fig. 3. Msi1 is elevated in TRAIL-resistant cells. HCC cell lines were treated with or without TRAL for 4 h. Total RNA was extracted to perform real-time PCR analysis of CD133, Oct4, Nanog, Sox2 (A) and Msi1 (B). (C) Msi1 mRNA was detected in LH86, LH86-TR, HLCZ01 and Huh7 cells. Error bars represented S.D. from triplicate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ verse control non-treated cells. (D) Msi1 protein was determined by Western blot analysis.

TRAIL, we monitored the accumulation of cell populations in early stage of apoptosis with annexin-V staining and the caspase-dependent cleavage of PARP. Annexin-V staining showed that TRAIL induced 20% apoptotic cells in LH86 (Fig. 1C), whereas LH86-TR cells were resistant to TRAIL (7%). The PARP and caspase-8 assay support the findings (Fig. 1D).

3.2. Pro-survival and apoptosis signaling in TRAIL-sensitive (LH86) versus TRAIL-resistant (LH86-TR) liver cancer cells

Our previous study have found that the intrinsic apoptotic pathway was activated in the TRAIL sensitive LH86 cells after treatment with TRAIL [11], so we investigated whether this signaling pathway was intact in LH86-TR cells. As shown in Fig. 2A and B, treatment with TRAIL induced the increase of Bid activity as well as caspase-9 and caspase-3 cleavage in a dose-dependent manner in both cells. However, both caspases (caspase-9 and caspase-3) and Bid activity in LH86-TR cells were markedly lower than that of parental cells. The induction of mitochondrial-dependent apoptotic pathway protein NOXA was also increased in LH86 cells compared to in LH86-TR cells. These results suggest that the intrinsic apoptotic pathway is disrupted in LH86-TR cells.

Previous study has suggested that STAT3 activation status may play an important role in mediating TRAIL resistance in HCC cells [12]. In this regard, we examined phosphorylated STAT3 in both LH86 and LH86-TR cells and found that p-STAT3 existed only in IFN-α treated cells. TRAIL did not induce STAT3 phosphorylation in these two cell lines (Fig. 2C). To gain further insight on the molecular basis of TRAIL resistance, we analyzed the expression of several other oncogenes and tumor suppressors following treatment with various concentrations of TRAIL. The examined genes such as BAX, p-PTEN, p-P38, k-RAS, Bcl-2 and Bcl-xl were not markedly changed in both cells after treated by TRAIL, the anti-apoptotic protein MCL-1 was elevated in LH86-TR cells (Fig. 2D). In contrast, the phosphorylation level of AKT was decreased dependent on the increasing TRAIL concentrations, the basal level of activated AKT in LH86-TR cells were much lower than in parental LH86 cells. The differential activation of AKT is not responsible for the acquisition of TRAIL resistance, because specific inhibition of AKT by LY294002 only slightly improved TRAIL-induced apoptosis (Fig. 2E). We also examined the expression level of TRAIL death receptors by using real-time PCR and Western blot analysis. No differences in expression levels of both death receptors (DR4 and DR5) and decoy receptors (DcR4 and DcR5) were observed between the parental LH86 and the resistant LH86-TR cells (Fig. 2F and G).

3.3. Stem cell marker Msi1 is elevated in TRAIL resistant liver cancer cells

Cancer stem cells (CSCs) subpopulation will be enriched in chemotherapeutic reagents selected drug-resistant cancer cells [9,13–15]. Next, we investigated if cancer stem cell markers and pluripotency factors increased in TRAIL-resistant cells. We first compared the expression of CD133 between LH86 and LH86-TR cells with TRAIL treatment. CD133 expression level in LH86-TR cells was lower than in LH86 cells. TRAIL induced a little decrease of CD133 in both cells. The stem cell transcription factors including Oct4, Nanog, and Sox2 stem cell transcription factors were lower in LH86-TR cells than in parental cells (Fig. 3A). Interestingly, though short time treatment with TRAIL reduced Msi1 expression in both cells, there was an over 10-fold increase of Msi1 in LH86-TR cells (Fig. 3B). To further confirm that Msi1 was increased in TRAIL-

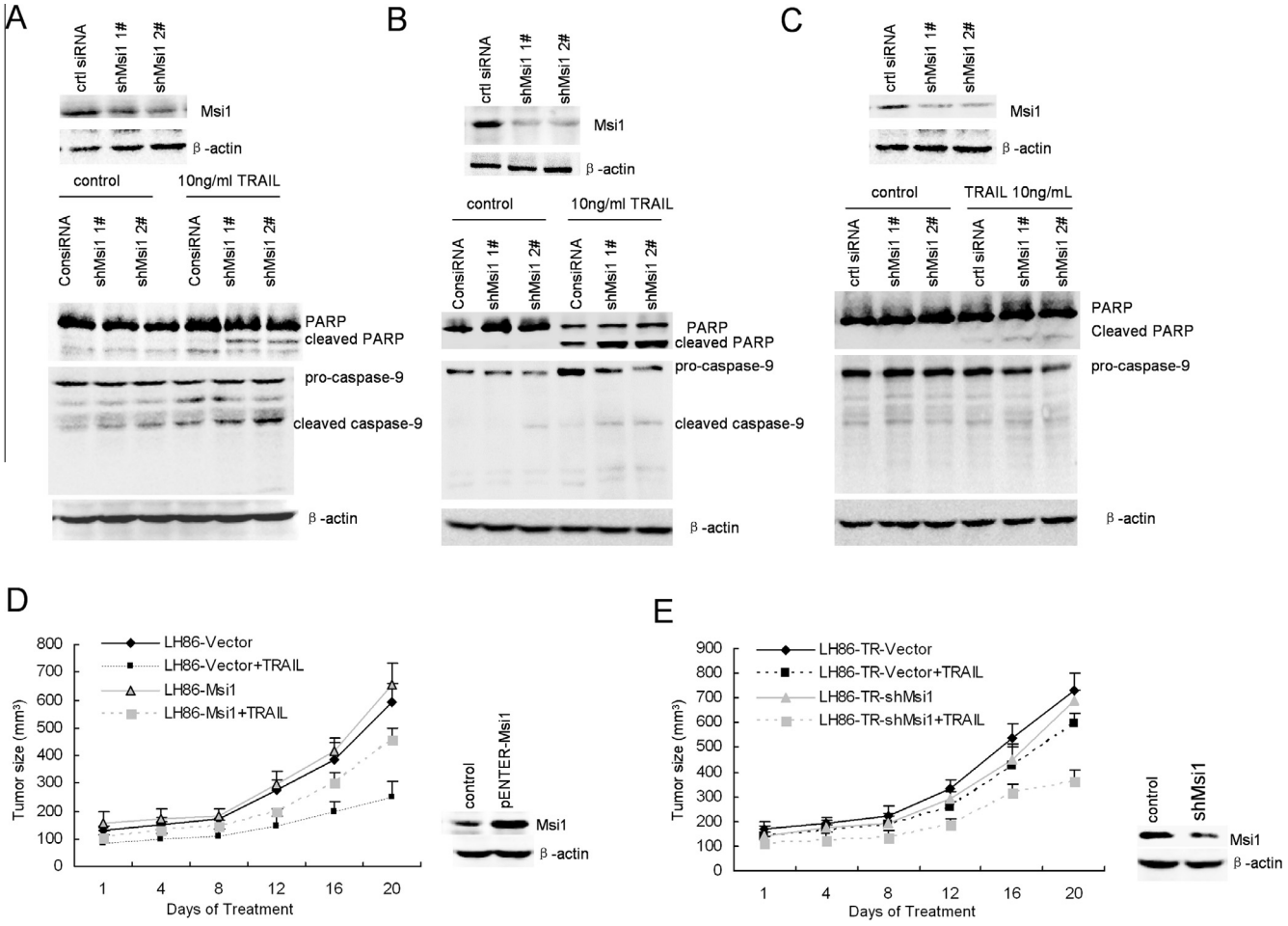

Fig. 4. Msi1 depletion restores TRAIL-responsiveness to TRAIL-resistant cells. LH86-TR (A), Huh7 cells (B) and HLCZ01 (C) cells were transiently transfected with indicated shRNA for 48 h and subsequently stimulated with TRAIL at different concentrations. Representative Western blot of knockdown efficiency and active form of caspase-9 and PARP were shown. (D) In LH86-Msi1 implanted nude mouse, TRAIL showed lower inhibition effects on tumor growth. Msi1 was overexpressed in LH86 cells (right panel). (E) In LH86-TR-shMsi1 implanted nude mouse, TRAIL showed higher inhibition effects on tumor growth. Msi1 knockdown efficacy in LH86-TR cells was shown in right panel.

resistant cells, we compared Msi1 expression between sensitive LH86 and resistant LH86-TR, Huh7, HLCZ01 cells. As shown in Fig. 3C, Msi1 level was much higher in resistant cells than in sensitive cells. The Western blot assay confirmed the findings (Fig. 3D). These data suggested that Msi1 may play an important role in TRAIL resistance.

3.4. Msi1 mediates TRAIL sensitivity of HCC cells in vitro and in vivo

To evaluate the effect of Msi1 knockdown on TRAIL-induced apoptosis in HCC cell lines, we used shRNA to silence Msi1 expression. ShRNA knockdown reduced Msi1 protein expression compared to control shRNA-transfected cells (Fig. 4A–C). Inhibition of Msi1 increased TRAIL-induced cleavage of PARP level as well as caspase-9 activation in LH86-TR cells (Fig. 4A). Similarly, Huh7 and HLCZ01 cells cultured in the presence of TRAIL showed higher PARP and caspase-9 activation in Msi1 knockdown cells compared to control shRNA-transfected cells (Fig. 4B and C). These results suggest that Msi1 inhibition sensitizes HCC cells to TRAIL through intrinsic apoptotic pathway activation. To test whether Msi1 regulates TARIL-induced apoptosis in vivo, we overexpressed Msi1 in LH86 cells or silenced it in LH86-TR cells. After selected with antibiotics for 2 weeks, stable-Msi1 expressing LH86 cells and stable-Msi1 knockdown LH86-TR cells were obtained (Fig. 4D and E). Then these cells were implanted into the immunodeficiency nude mice, respectively. TRAIL treatment was initiated when the tumor size reached to about 100 mm^3. In nude mice, TRAIL treatment has little inhibition effect on tumor growth in Msi1 overexpressing LH86 cells compared with control groups (Fig. 4D). Conversely, knockdown of Msi1 sensitized LH86-TR cells to TRAIL treatment (Fig. 4E). All these data suggested that Msi1 mediates TRAIL sensitivity of HCC cells in vitro and in vivo.

3.5. ERK signaling is involved in Msi1-mediated TRAIL resistance of HCC cells

Previous studies show that Msi1 reduction leads to a decrease in the AKT signaling activity in Daoy cells [7]. Though AKT activity is decreased in the LH86-TR cells which acquired resistance to TRAIL (Fig. 2E), we wonder if Msi1 knockdown could reduce AKT activity in this cell line. As shown in Fig. 5A, AKT activity was completely inhibited by treated with LY294002 but not changed after treatment with Msi1-targeted shRNA in this cell line. Interestingly, we found that ERK activity and its downstream gene MCL-1 were decreased in Msi1-targeted shRNA transfected cells compared with control shRNA transfected cells. The basal level of p-ERK was higher in LH86-TR cells than in LH86 cells (Fig. 5B). To further confirm this finding, LH86 cells were transfected with full-length Msi1 cDNA construct and treated by TRAIL. pERK and MCL-1 levels were increased in Msi1 overexpressing cells compare

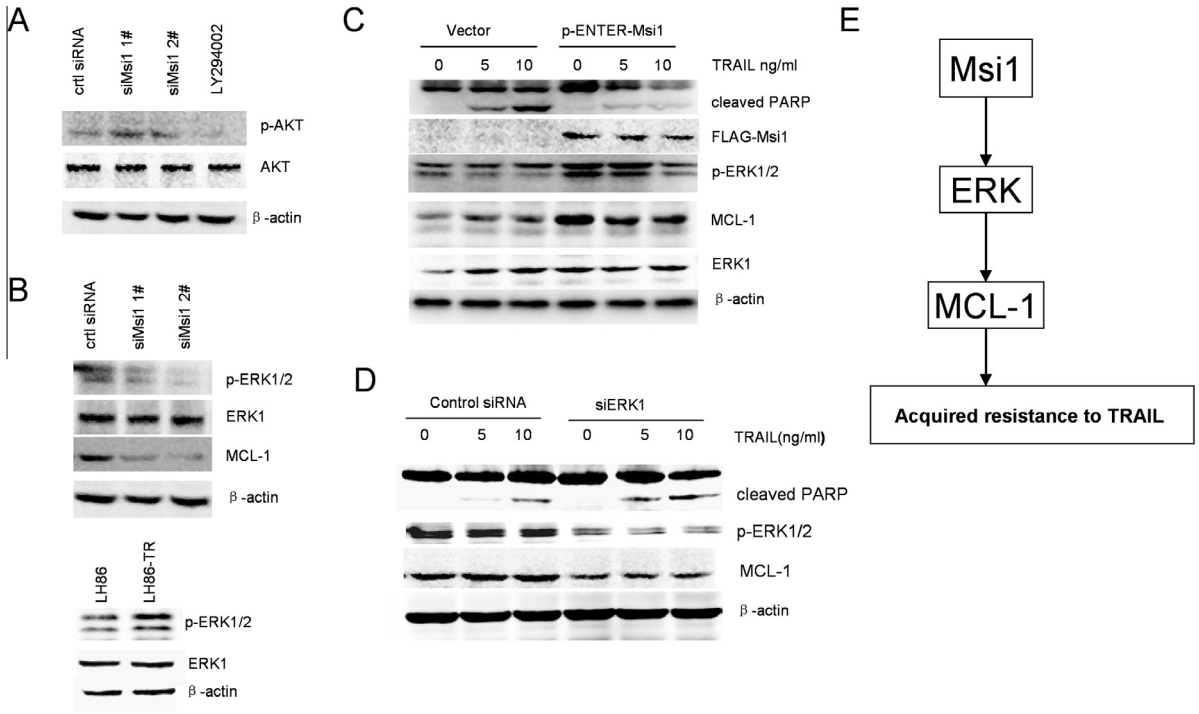

Fig. 5. Msi1 regulates ERK activation. (A) Msi1 knockdown had no effects on AKT activation. (B) Inhibition of Msi1 decreased ERK activation and MCL-1 expression in LH86-TR cells (up). The basal level of p-ERK was examined by Western blot assay (down). (C) Overexpression of Msi1 increased ERK activation and MCL-1 expression and inhibited TRAIL-induced apoptosis in LH86 cells. Apoptosis was measured by PARP cleavage. (D) Knock down of ERK sensitizes LH86-TR cells to TRAIL through downregulation of MCL-1. (E) A schematic diagram showing the proposed Msi1 signaling cascade that regulates the acquired resistance to TRAIL in HCC cells.

with control cells (Fig. 5C). TRAIL-induced cleavage PARP was lower in Msi1 overexpressing cells than in control cells (Fig. 5C). To investigate whether inhibition of ERK could enhance TRAIL-induced apoptosis, we knock down ERK in LH86-TR cells by using specific siRNA. As shown in Fig. 5D, ERK and MCL-1 were inhibited and activating form of PARP was increased in ERK knockdown cells. These results indicated that Msi1 inhibits TRAIL-induced apoptosis by activating ERK and inhibition of ERK can sensitize TRAIL-resistant cells to TRAIL.

4. Discussion

Drug resistance is a common phenomenon and a major obstacle to most cancer therapies, including TRAIL-based therapy. Many tumor cells display resistance to TRAIL and susceptible tumor cells may acquire resistance mechanisms due to adaptive changes in response to therapy and selection of survival phenotypes. Both congenital and acquired TRAIL resistance mechanisms have been reported in some cell lines. STAT3 are involved in cell proliferation and survival and frequently activated. Sorafenib inhibits STAT3 activation and overcomes TRAIL resistance in pancreatic cancers and liver cancers [12,16,17]. These results indicate that STAT3 are involved in primary TRAIL resistance in tumor cells, although we did not observe the different activation level of STAT3 between LH86 and LH86-TR cells. Our previous study showed that ISG12a modulates TRAIL sensitivity and is regulated by miR-942. ISG12a basal level was lower in TRAIL resistant cells (such as Huh7 and HLCZ01) than in TRAIL sensitive cells (LH86) [11]. Increased c-FLIP and Mcl-1 expression confers acquired resistance to TRAIL-induced cytotoxicity in lung cancer cells [18]. In colon adenocarcinoma cells, cell surface galectin-3 confers resistance to TRAIL by impeding trafficking of death receptors [19]. The acquired TRAIL resistance mechanism is controversial and rarely studied in HCC cells.

In the present study, we demonstrated that TRAIL-sensitive HCC cell line LH86 became resistant to TRAIL-induced apoptosis after prolonged treatments with TRAIL. The intrinsic apoptotic pathway was not activated in resistant cells compared to in sensitive cells after treatment with TRAIL. Though increased AKT activity contributes to TRAIL resistance in lung and breast cancer cells [18,20], we found decreased AKT activity in LH86-TR cells. Suppressing AKT activity with LY294002 did not sensitize LH86-TR cells to TRAIL-induced cytotoxicity. These findings suggested that AKT may not play a functional role in acquired TRAIL resistance in HCC cells.

It has been reported that Msi1 modulates cell proliferation and apoptosis in various cancer cells [6,7,21]. In an in vivo study, Msi1 knock down inhibits tumor growth [22]. Msi1 regulates cell survival by activating Wnt signaling pathway in liver cancer cells [10]. Msi1 is elevated in selected sorafenib resistant HCC cell lines. Increased Msi1 expression is associated with enhanced cellular migration and invasion [9]. In our study, we found that Msi1 was the only increased pluripotency factor among others in LH86-TR. Knock down of Msi1 with specific shRNA sensitized LH86-TR cells to TRAIL-induced apoptosis. Overexpression of Msi1 blocked TRAIL-induced apoptosis in LH86 cells. As a potential cancer stem cell marker, CD133 confers drug resistance in cancer cells [23]. The level of CD133 is lower in LH86-TR cells than in parental cells. It would be possible that cancer stem cells are not enriched in LH86-TR cells and are not the main reason for TRAIL resistance in this cell line. Msi1 exerts their regulatory functions by binding the 3′untranslated region of mRNA of the target and inhibits its expression [24]. Whether Msi1 regulates CD133 expression by binding the mRNA of CD133 needs to be further studied.

Previous study demonstrated that breast cancer cells acquire resistance to TRAIL through ERK activation [25]. ERK activation inhibits the processing of caspase-8 and Bid, thereby turning off the mitochondrial amplification loop [26,27]. Previous studies

showed that Mcl-1 protein expression is enhanced due to ERK activation [28–30]. In our study, we observed that activation of ERK and protein level of MCL-1 decreased in Msi1 knockdown LH86-TR cells whereas increased in Msi1 overexpressing LH86 cells. Furthermore, inhibiting ERK activation with siRNA overcomes the acquired TRAIL resistance of LH86-TR cells. These findings suggest that combination ERK inhibitor or specific siRNA with TRAIL can sensitize TRAIL-resistant cells to TRAIL-induced apoptosis through downregulation of MCL-1. A genomic analysis of Msi1 downstream targets found many cancer-related genes [24]. Whether these Msi1 targeted genes are associated with TRAIL sensitivity needs to further studied. Several miRNAs have been found to affect Msi1 expression [31]. It would be interesting to investigate whether these miRNAs are decreased in TRAIL-resistant cells and regulate TRAIL sensitivity by modulating Msi1 expression. A recent study showed that Msi1 can serve as a diagnostic marker and a potential therapeutic target in lung cancer cells [32], whether it has a similar role in HCC cells remain to be explored.

In summary, we found that Msi1 is elevated in TRAIL-resistant cells and confers TRAIL resistance through ERK activation. The upregulated MCL-1 after ERK activation may also contributes to acquire resistance to TRAIL in HCC cells (Fig. 5E). The inverse correlation of Msi1 expression level and TRAIL sensitivity indicated that Msi1 can serve as a TRAIL response marker with important implications in designing new therapeutics.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by 2009ZX10004-312 from National Science and Technology Major Project, Grant 81271885 from National Natural Science Foundation of China and NCET-09-0339 from Program for New Century Excellent Talents in University (H.Z.).

References

[1] Marquardt, J.U. and Thorgeirsson, S.S. (2014) SnapShot: hepatocellular carcinoma. Cancer Cell 25 (4). 550-550.
[2] Gonzalvez, F. and Ashkenazi, A. (2010) New insights into apoptosis signaling by Apo2L/TRAIL. Oncogene 29 (34), 4752–4765.
[3] Lemke, J., von Karstedt, S., Zinngrebe, J., et al. (2014) Getting TRAIL back on track for cancer therapy. Cell Death Differ. 21, 1350–1364.
[4] Wiezorek, J., Holland, P. and Graves, J. (2010) Death receptor agonists as a targeted therapy for cancer. Clin. Cancer Res. 16, 1701–1708.
[5] Okano, H., Kawahara, H., Toriya, M., et al. (2005) Function of RNA-binding protein Musashi-1 in stem cells. Exp. Cell Res. 306 (2), 349–356.
[6] Sanchez-Diaz, P.C., Burton, T.L., Burns, S.C., et al. (2008) Musashi1 modulates cell proliferation genes in the medulloblastoma cell line Daoy. BMC Cancer 8 (1), 280.
[7] Muto, J., Imai, T., Ogawa, D., et al. (2012) RNA-binding protein Musashi1 modulates glioma cell growth through the post-transcriptional regulation of Notch and PI3 kinase/Akt signaling pathways. PLoS One 7 (3), e33431.
[8] Wang, X.Y., Yin, Y., Yuan, H., et al. (2008) Musashi1 modulates mammary progenitor cell expansion through proliferin-mediated activation of the Wnt and Notch pathways. Mol. Cell. Biol. 28 (11), 3589–3599.
[9] Chow, A.K.M., Ng, L., Lam, C.S.C., et al. (2013) The enhanced metastatic potential of hepatocellular carcinoma (HCC) cells with sorafenib resistance. PLoS One 8 (11), e78675.
[10] Chen, K., Gao, Q., Zhang, W., et al. (2015) Musashi1 regulates survival of hepatoma cell lines by activation of Wnt signalling pathway. Liver Int. 35 (3), 986–998.
[11] Liu, N., Zuo, C., Wang, X., et al. (2014) miR-942 decreases TRAIL-induced apoptosis through ISG12a downregulation and is regulated by AKT. Oncotarget 5 (13), 4959.
[12] Chen, K.F., Tai, W.T., Liu, T.H., et al. (2010) Sorafenib overcomes TRAIL resistance of hepatocellular carcinoma cells through the inhibition of STAT3. Clin. Cancer Res. 16 (21), 5189–5199.
[13] Gupta, P.B., Onder, T.T., Jiang, G., et al. (2009) Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 138 (4), 645–659.
[14] Levina, V., Marrangoni, A.M., DeMarco, R., et al. (2008) Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. PLoS One 3 (8), e3077.
[15] Bertolini, G., Roz, L., Perego, P., et al. (2009) Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc. Natl. Acad. Sci. 106 (38), 16281–16286.
[16] Huang, S. and Sinicrope, F.A. (2010) Sorafenib inhibits STAT3 activation to enhance TRAIL-mediated apoptosis in human pancreatic cancer cells. Mol. Cancer Ther. 9 (3), 742–750.
[17] Abdulghani, J., Allen, J.E., Dicker, D.T., et al. (2013) Sorafenib sensitizes solid tumors to Apo2L/TRAIL and Apo2L/TRAIL receptor agonist antibodies by the Jak2-Stat3-Mcl1 axis. PLoS One 8 (9), e75414.
[18] Wang, X., Chen, W., Zeng, W., et al. (2008) Akt-mediated eminent expression of c-FLIP and Mcl-1 confers acquired resistance to TRAIL-induced cytotoxicity to lung cancer cells. Mol. Cancer Ther. 7 (5), 1156–1163.
[19] Mazurek, N., Byrd, J.C., Sun, Y., et al. (2011) Cell-surface galectin-3 confers resistance to TRAIL by impeding trafficking of death receptors in metastatic colon adenocarcinoma cells. Cell Death Differ. 19 (3), 523–533.
[20] Xu, J., Zhou, J.Y., Wei, W.Z., et al. (2010) Activation of the Akt survival pathway contributes to TRAIL resistance in cancer cells. PLoS One 5 (4), e10226.
[21] Götte, M., Greve, B., Kelsch, R., et al. (2011) The adult stem cell marker Musashi-1 modulates endometrial carcinoma cell cycle progression and apoptosis via Notch-1 and p21WAF1/CIP1. Int. J. Cancer 129 (8), 2042–2049.
[22] Sureban, S.M., May, R., George, R.J., et al. (2008) Knockdown of RNA binding protein musashi-1 leads to tumor regression in vivo. Gastroenterology 134 (5), 1448–1458.
[23] Zhang, L., Li, H., Ge, C., et al. (2014) Inhibitory effects of transcription factor Ikaros on the expression of liver cancer stem cell marker CD133 in hepatocellular carcinoma. Oncotarget 5 (21), 10621.
[24] de Sousa, Abreu R., Sanchez-Diaz, P.C., Vogel, C., et al. (2009) Genomic analyses of musashi1 downstream targets show a strong association with cancer-related processes. J. Biol. Chem. 284 (18), 12125–12135.
[25] Lee, T.J., Lee, J.T., Park, J.W., et al. (2006) Acquired TRAIL resistance in human breast cancer cells are caused by the sustained cFLIP and XIAP protein levels and ERK activation. Biochem. Biophys. Res. Commun. 351 (4), 1024–1030.
[26] Söderström, T.S., Poukkula, M., Holmström, T.H., et al. (2002) Mitogen-activated protein kinase/extracellular signal-regulated kinase signaling in activated T cells abrogates TRAIL-induced apoptosis upstream of the mitochondrial amplification loop and caspase-8. J. Immunol. 169 (6), 2851–2860.
[27] Mandal, R., Raab, M., Matthess, Y., et al. (2014) PERK 1/2 inhibit Caspase-8 induced apoptosis in cancer cells by phosphorylating it in a cell cycle specific manner. Mol. Oncol. 8 (2), 232–249.
[28] Wang, B., Ni, Z., Dai, X., et al. (2014) The Bcl-2/xL inhibitor ABT-263 increases the stability of Mcl-1 mRNA and protein in hepatocellular carcinoma cells. Mol. Cancer 13 (1), 98.
[29] Wang, B., Chen, L., Ni, Z., et al. (2014) Hsp90 inhibitor 17-AAG sensitizes Bcl-2 inhibitor (-)-gossypol by suppressing ERK-mediated protective autophagy and Mcl-1 accumulation in hepatocellular carcinoma cells. Exp. Cell Res. 328 (2), 379–387.
[30] Chen, C.H., Chen, M.C., Wang, J.C., et al. (2014) Synergistic interaction between the HDAC inhibitor, MPT0E028, and sorafenib in liver cancer cells in vitro and in vivo. Clin. Cancer Res. 20 (5), 1274–1287.
[31] Vo, D.T., Qiao, M., Smith, A.D., et al. (2011) The oncogenic RNA-binding protein Musashi1 is regulated by tumor suppressor miRNAs. RNA Biol. 8 (5), 817–828.
[32] Wang, X.Y., Yu, H., Linnoila, R.I., et al. (2013) Musashi1 as a potential therapeutic target and diagnostic marker for lung cancer. Oncotarget 4 (5), 739.